Phase Transitions in the Nucleus

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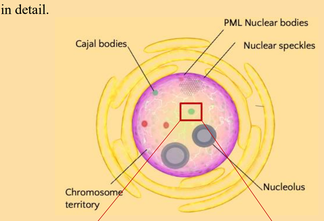
Abstract:

Eukaryotic cells organize its cellular components into organelles for improved cellular efficiency. Compartmentalization enables saturation of reaction components in one location to increase metabolic reaction kinetics. However, some cellular compartments are not bound by membranes. As an alternative to membrane encapsulation, cells form membranelles organelles via the process of liquid-to-liquid phase separation (LLPS). LLPS-driven compartments, also referred to as biomolecular condensates, emerge from weak transient interactions between intrinsically disordered proteins (IDPs) and other biomolecules, primarily RNA and DNA. Self-associating IDPs are key regulators of LLPS as they lack a 3-dimensional protein structure due to the abundance of charged, hydrophilic amino acids within its linear protein sequence. The unique characteristics of IDPs enable them to maintain weak multivalent interactions that result in selective concentration of free-floating cytoplasmic molecules. Under preferable physical conditions, the localized molecules transition from dilute to molecule-rich phase forming a chemically unique gel-like condensate capable of performing biological functions. Biomolecular condensates are prevalent inside the nucleus of eukaryotic cells and range in size from relatively large nucleoli to tiny nuclear speckles. The advances in recent research showed that phase separated states hold a central role in nucleus organization and functionalization; however, the intricacies of this topic are yet to be explored. In this literature review and the accompanying poster, three instances of biological phase transitions inside the nucleus, including heterochromatin gene regulation, transcriptional regulation by RNA polymerase II and ribosome assembly, will be discussed in detail. Specifically, we interpret the molecular mechanisms and biophysical aspects that form, segregate and localize LLPS condensates in the nuclear space to perform each of three nuclear functions. The purpose of this review is to summarize the existing information on the topic area and build a comprehensive understanding of the complex concept by presenting the information in a structured and coherent manner.

Introduction

Phase Separation

Eukaryotic cells organize its cellular components into membrane-bound organelles to improve cellular efficiency of a cell by concentrating key reaction components in one location. In addition to membrane-enclosed organelles, cellular cytoplasm of eukaryotic cells contains chemically variable compartments that lack a membrane. These structures, broadly referred to as **biomolecular condensates**, are dynamic entities that range in size from 0.1- 4 um in diameter and allow selective concentration of molecules to perform various biological functions. In this poster, biomolecular condensates inside the nucleus will be looked at in detail.



- Membraneles organelles assemble through the process called liquid-liquid phase separation (LLPS).
- LLPS allows rapid concentration of molecules that results in physical shift from liquid to a liquid-like dense phase.

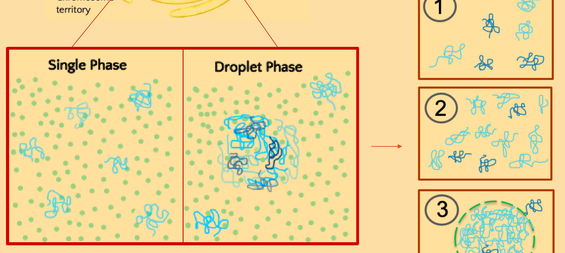
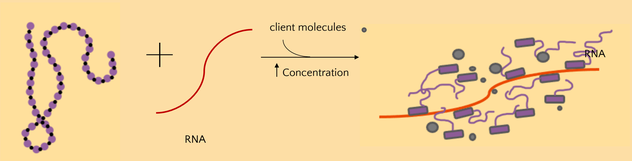


Figure 1. The schematic representation of the assembly of a biomolecular condensate (Cajal body) inside the nucleus.

Regulation of Condensate Assembly

Biomolecular condensates are composed of many different types of molecules, but the assembly of the compartment is primarily driven by RNA and intrinsically disordered proteins (IDPs). Intrinsically disordered proteins lack a 3-D protein shape due the abundance of charged, hydrophilic and disorder promoting amino acids (A, R, G, Q, S, P, E and K) that prevent proteins from forming a hydrophobic core. IDPs also contain short repeated sequence elements that maintain multiple interactions with RNA and other molecules.



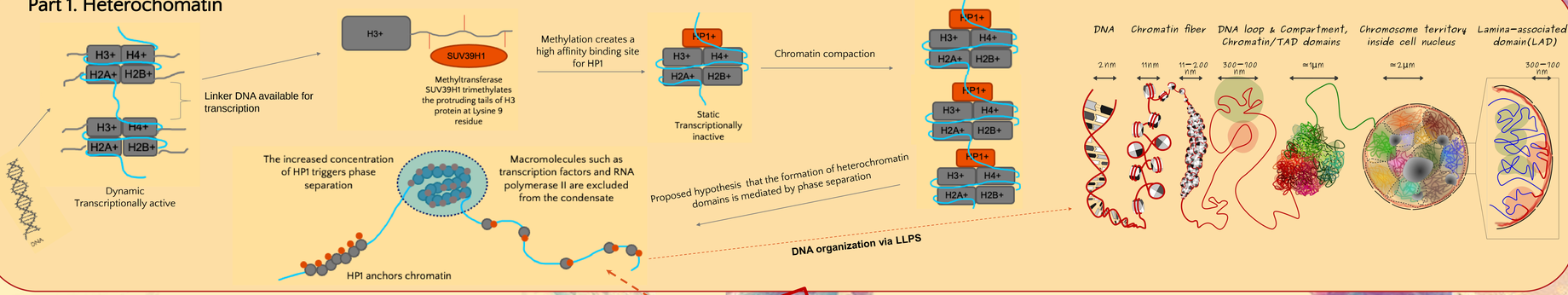
Intrinsically disordered protein (IDP) — Multivalent interactions

Why is it relevant?

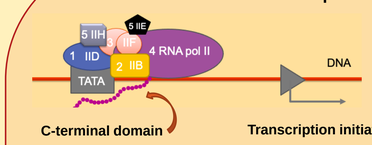
- The aberrant phase transitions were shown to be associated with pathologies such as neurodegenerative diseases, diabetes and cancer.
- Understanding the molecular mechanisms of LLPS driven condensates would enable scientists to potentially mitigate disease.

Instances of Phase Transition in the Nucleus

Part 1. Heterochomatin

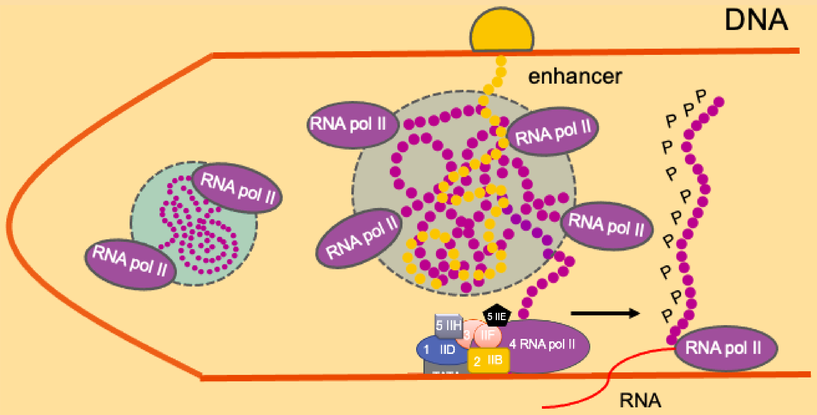


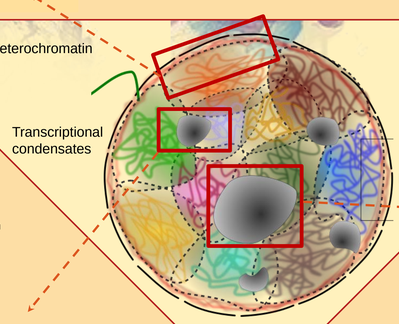
Part 2: Transcriptional regulation.



- Disordered sequence
- Contains repeated heptapeptides (YSPTSPS)
- Provides multiple phosphorylation sites

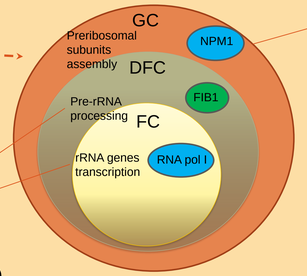
- The transition from transcription initiation to elongation involves a redistribution of RNAPII from initiation-type condensates into elongation-type condensates
- This transition is a rate limiting step that results in pausing or transcription halting
- The phosphorylation of the CTD provides the driving force for transcription initiation by weakening the interactions that hold the condensate components together



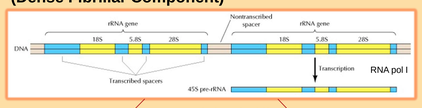


Part 3: Ribosome assembly

In the nucleus, nucleoli are the sites of ribosome synthesis and assembly that form around chromosomes that contain stretches of repeating rDNA sequences, known as nuclear organizer regions (NORs). The multi-layered structure of the nucleus is thought to facilitate assembly line processing of rRNA by undergoing LLPS.



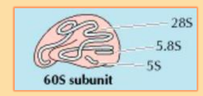
1. Transcription (Fibrillar Centre) and Processing (Dense Fibrillar Component)



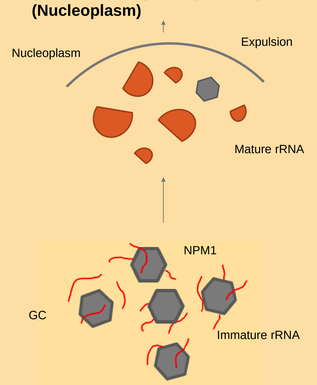
snoRNPs (U3 and U22)

18S rRNA
+ ribosomal proteins

5.8 and 28S rRNA + 5S (synthesized outside the nucleus)
+ ribosomal proteins



2. Preribosomal subunits assembly (Granular Component) and export (Nucleoplasm)



- FIB1 and NMP1 were experimentally shown to phase separate in vitro
- FIB1 assembly primarily depends on protein concentration, whereas NPM1 requires high concentrations of both protein and rRNA
- Continuous expression of rRNA within FC causes a flux of rRNA through the DFC into the GC, hence inducing its assembly

Conclusion

- The discovery of LLPS has significantly impacted our current understanding of cellular compartmentalization in eukaryotic cells. The three processes of nuclear assemblies discussed above are likely to represent only a small subset of LLPS compartments present inside the nucleus. In fact, it is possible that high abundance of chemically variable nuclear condensates is key to cellular adaptability to environmental and developmental changes.
- Future experiments that focus on biophysical properties of biomolecular condensates both *in vitro* and *in vivo* might provide insights into complex processes such as regulation of genome as well as introduce new approaches to disease therapy for a broad spectrum of neurological disorders and cancer. Furthermore, with the recent evidence that demonstrated the ability of certain bacterial proteins to undergo phase separation, it has also been proposed that biomolecular compartments functioned as early protocells in the beginning of life. It appears that functional capabilities of LLPS are only beginning to be unravelled.
- Even though LLPS condensates have become a default explanation for compartmentalization of intracellular environments, it is still unclear how valid the evidence for *in vivo* LLPS is. Current biomolecular tools are arguably suitable for examining condensate assembly *in vivo*, therefore, better and more creative assays for LLPS diagnosis are needed. These assays must directly illustrate the effects of concentration, temperature, and intermolecular interactions on the LLPS compartments in question.